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# Exploring The Role Of Dopamine Circuits In Eating Behavior

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# **Exploring the Role of Dopamine Circuits in Eating Behavior**

A Thesis Submitted to the

Yale University School of Medicine

In Partial Fulfillment of the Requirements for the

Degree of Doctor of Medicine

By

Kara Elizabeth Furman

2017

## **Abstract**

In its simplest terms, obesity is a disease of inappropriate food intake. While current treatment of obesity focuses on changing behavior, few successful models of behavior modification have been developed. Even those treatments that are successful in the short-term, have failed to demonstrate lasting effect.

The problem of over-eating, then, needs to be studied in terms of basic motivational programs that are encoded by the brain's reward system.

Understanding the way in which reward system activity directs eating behavior will allow more sophisticated and efficacious treatment options in the future.

Several studies have focused on the nucleus accumbens (NAc) as an important regulator of motivation and reward seeking. There are two primary populations of neurons in the NAc at work in modulating motivation and reward-related effort. Several studies have given insight into what each of these may be doing to direct food intake. The present study expands on this body of knowledge using a novel technique to accomplish cell-type-specific assessment of neural activity during feeding behavior.

## Acknowledgements

I would like to thank my mentor Ralph J. DiLeone for giving me guidance in how to think scientifically, latitude to develop my own research questions and discover my capabilities, and continuous support in times of success and struggle.

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## **1. Obesity and Eating: Clinical Treatment and Scientific Understanding**

Food and nutrition are increasingly recognized as primary determinants of health and disease. While proper nutrition is essential to normal growth and development, over-consumption of calorie-dense foods high in simple carbohydrates, salt and saturated fat cause diseases like hypertension, diabetes and obesity (associated with these and multiple other health problems). Dramatically increased rates of obesity in the United States (from 15% adults in 1990 to 35% adults in 2014) has prompted study of the pathophysiology of weight gain from several disciplinary perspectives. From an economic standpoint, the social and medical impacts of obesity are significant. In 2008, the estimated medical cost of obesity-related dysfunction was \$147 billion dollars, and persons with obesity incur 42% higher medical costs than average (Finkelstein, Trogdon, Cohen, & Dietz, 2009). It is fair, then, to call rising rates of obesity a healthcare crisis that needs new tools to address treatment and prevention. While genetic, metabolic, hormonal and immune system dysfunction are all associated with obesity, in its simplest terms, obesity results from energy imbalance: too much intake and too little expenditure.

Logically, behavior modification is first-line treatment for overweight and obesity. Despite such therapy, patients often fail to achieve lasting change or avoid negative health consequences of these disorders. Reasons that behavior modification fails are myriad. As patients revert to problematic habits, it becomes clear that effective behavioral strategies require a better understanding of the

motivational drives and decision-making processes involved in food choice and food intake. An emerging field in molecular psychiatry provides a new perspective on eating behavior that focuses on the effect of food on the reward system and gives insight to new avenues for behavioral and pharmacologic treatment of disordered eating. A review of current treatment practices underscores the need for further research into the role of the brain's reward circuit in directing eating behaviors.

## **1.1 Treatment Approaches for Obesity**

### **1.1.1 Lifestyle Modification**

In the primary care clinic, lifestyle modification is first-line treatment for overweight and obesity. Obesity is defined by Body Mass Index (BMI) greater than 30 (BMI is an index that uses height, weight and sex to quantify body fat tissue). This is not a perfect measure, however it is widely accepted and used to stratify patients. BMI of 19-25 is classified as normal, and BMI 25-30 is classified as overweight). The current standard of care in obesity treatment is first to enact a behavioral therapy program. Intensive behavioral therapy is most effective (Tate & Wing, 2000; Wadden & Butryn, 2003; Wadden, McGuckin, Rothman, & Sargent, 2003) and is generally carried out over a period of 6-12 months. Most programs start with weekly sessions for the first 4-6 weeks, followed by gradual spacing out of sessions from bi-weekly, to monthly. Long-term follow-up intervals

vary between programs but have demonstrated effectiveness up to 2 years. In general, models for behavioral interventions aim to reduce daily caloric intake and increase daily exercise using a cadre of behavioral and cognitive techniques to help patients maintain healthy habits (Berkel, Poston, Reeves, & Foreyt, 2005; Carvajal, Wadden, Tsai, Peck, & Moran, 2013). Calorie restriction diets are usually modest, 1200 kcal/day for women, and 1500 for men. Patients are given healthy food options and advised to avoid calorie-dense, high-sugar and high-fat, food sources (Berkel et al., 2005; Fabricatore & Wadden, 2006). There is no consensus on recommendations for how to balance macronutrients (i.e. fat, protein, carbohydrates) at each meal and throughout the day. Exercise goals are set at 30 minutes of moderate cardiovascular challenge per day (e.g. walking 4.0 mph). Patients will hopefully achieve excess calorie burn of about 1000 kcals per week. Several studies have demonstrated success using behavioral modification with most patients achieving 5-10% weight loss (Fabricatore et al., 2011). While this degree of weight loss may not normalize BMI, in 2003, a landmark study the Look AHEAD trial, demonstrated that even a 7% weight loss has clinically significant benefits on cardiovascular health and glucose tolerance (Ryan et al., 2003). Therefore, intensive behavioral programs are largely seen as a low-cost intervention to achieve clinically meaningful weight loss.

In 2011, these promising data prompted the Centers of Medicare and Medicaid Services (CMS) to begin covering intensive behavioral therapy for treatment of obesity in primary care (Services, 2011). However, the model



supported by the CMS differs significantly from those that have been studied.

First, the CMS mandates that therapy be completed by primary care providers in the clinic rather than by specialized therapists. Second, although their model covers the same number of visits as most successful intensive therapy programs, CMS limits session time to 10-15 minutes rather than 60-90 minute sessions endorsed by successful weight loss programs (Force, 2003; Pagoto, 2012).

These differences in implementation have caused significant concern among primary care providers who often lack training in behavioral therapy. A year after the CMS policy change, the Society of Behavioral Medicine published its own policy brief commending the CMS decision to support behavioral therapy but noting “concern about the degree to which it can be feasibly implemented in its current form given the limitations in providers who are covered and the length of counseling visits being significantly shorter than in evidence-based protocols” (Pagoto, 2012). In addition, the intensive therapy schedule dramatically increases the time burden on already under-resourced clinics. Not surprisingly, preliminary evaluations of primary care-based interventions have demonstrated limited efficacy. A review of several primary care-based interventional models found an average weight loss of just 1-3 kg in 6-24 months of intervention (Carvajal et al., 2013). Success has been limited by infrequent and brief interactions between patient and caregivers, which are inadequate for establishing the momentum needed for meaningful change but may be all that can be achieved in busy primary care practices. A second limitation is that

sessions are not carried out by personnel trained in behavioral therapy—an essential feature of successful programs. More time is needed to fully assess the successes and constraints of the primary care model of obesity intervention. With this backdrop, what is most concerning about the treatment of obesity in both the primary care and intensive behavioral program settings is that long-term adherence to weight loss strategies is incredibly poor. Weight gain and relapse are almost inevitable.

Long-term follow-up of individuals who have successfully lost 10% of initial body weight shows that 80% have regained weight at one year (Wing & Phelan, 2005). Even in the most successful programs in which significant weight loss is achieved by most participants early on, as frequency and intensity of therapy declines, weight loss stops and the pounds gradually come back. Extended therapy augmented by telephone calls text or email check-ins can delay weight regain (Appel et al., 2011; Ely et al., 2008; Perri, 2014). Still, few patients can maintain weight loss for the 2-4 year benchmark that is associated with long-term successful weight management (Wing & Phelan, 2005).

### **1.1.2 Bariatric Surgery**

Failures in primary care and behavioral management of obesity have led to an increase in popularity and necessity of bariatric surgery. Because of the general risk associated with surgery (amplified by obesity), bariatric surgery is reserved for patients with BMI > 40 or > 35 with a significant medical co-morbidity.

There are several surgical procedures for weight loss, which have an overall 90% success rate defined by excess weight reduction of  $> 50\%$  in the first year (Spivak, Abdelmelek, Beltran, Ng, & Kitahama, 2012). Achieving this weight loss with behavioral therapy alone seems only possible with inpatient programs as seen on television shows like “The Biggest Loser,” in which participants are sequestered and placed on strict diet and exercise schedule as well as given group therapy for a period of weeks-months. Thus, bariatric surgery is currently the best treatment option for excess weight loss and resolution of metabolic syndrome in obese subjects who require greater than 5-10% weight reduction. Still, surgery does not eliminate the need for behavior therapy. Long-term success in these patients requires lasting lifestyle changes. The field of bariatric surgery has been very conscientious about mandating that patients demonstrate an ability to lose weight through lifestyle changes before surgery. However, surgeons have the same faulty behavioral intervention tools as primary care physicians.

This point is underscored by studies of long-term health outcomes of patients who have undergone Roux-en-Y gastric bypass (RYGB)—the most effective bariatric surgery. One study reviewed 10-year outcomes for 1,402 RYGB patients and found that at 18 months, patients reached a nadir in weight loss. At this point post-surgery, patients achieved 79% excess weight loss (EWL), and average BMI was reduced from 47 to 30. However, patients subsequently regained weight and at 12 years post-surgery, patients rebounded to 53% EWL

compared to pre-surgery body weight and average BMI was 37. In addition, at 10 years, there was a 19% recurrence in diabetes (Kothari, Borgert, Kallies, Baker, & Grover, 2016).

Weight regain after bariatric surgery is an issue gaining attention. Due to the high-initial cost and risk of this obesity treatment, surgery is only cost-effective if patients maintain weight loss and associated health gains in the long-term. Up to 60% of gastric-band patients experience weight regain (Magro et al., 2008). Factors associated with post-surgical regain include insufficient lifestyle modification and inadequate treatment of underlying eating disorders that contribute to obesity. Although immediately after surgery, most patients experience a decrease in appetite and food cravings, these return over time and patients may be ill-equipped to control their eating behaviors (Maleckas, Gudaityte, Petereit, Venclauskas, & Velickiene, 2016).

### **1.1.3 Cognitive Behavioral Therapy**

One theory about the lack of lasting success of behavioral therapy is that these interventions do not adequately address cognitive control mechanisms involved in making decisions around food. In 2010, Cooper et al posited that long-term failures in weight loss maintenance might be mitigated by employing cognitive behavioral therapy (CBT) aimed to increase patients' awareness of cognitive processes that hinder weight loss (e.g. stress, despair, unrealistic expectations). To investigate this idea, they conducted a 3-year longitudinal

study in which participants were assigned to receive traditional behavioral therapy (BT) or CBT. At the end of the study, the authors found that CBT had no effect on long-term weight loss when compared to traditional BT, and in fact, at three years, patients in both groups had regained almost all the weight lost during initial therapy. However, CBT did significantly improve patient's self-perception and reduce negative psychiatric symptoms (Cooper et al., 2010). It seems, then, that behavioral change is not simply a matter of lack of education or cognitive tools to deal with pathological eating.

## **1.2 Food Addiction**

Perhaps, a useful shift in perspective would reframe understanding of the primary dysfunctions involved in over-eating. Rather than focus on effort to exert greater executive control over food intake, we might think about ways in which foods can derail cognitive control systems. In other words, certain foods may have the capacity to alter an individual's neural circuitry in such a way that cognitive control is no longer feasible. In such cases, these foods will have become addictive, and patients may now require specialized intervention that targets foods as substances of abuse.

### **1.2.1 Validating Food Addiction**

A growing body of research has demonstrated parallels between effects of drugs of abuse and certain foods that are high in sugar and/or fat on addiction-

like behavior. According to the DSM-IV-TR, there are seven features of behavior that constitute addiction. Several of these can be modeled in animals fed high-fat or high-sugar diets. Most prominent behaviors include overuse, tolerance, compulsive intake, and even withdrawal. In 2008 Avena et al. provided convincing evidence that animals given intermittent access to sugar developed a “sugar addiction” phenotype in which animals displayed bingeing behaviors, as well as evidence of withdrawal, craving, cross-sensitization and gateway effect in which animals are more vulnerable to excessive alcohol intake when remaining abstinent from sugar (Avena, Rada, & Hoebel, 2008).

Research on the similarities between addiction and overeating has led to the question of whether people can suffer from “food addiction.” Work by Ashley Gearhardt at Yale opened this field of study with the development of the Yale Food Addiction Scale in 2009. Prior to the development of this scale the idea of food addiction had been studied based on self-diagnosis and varying definitions. The YFAS has now been validated in several studies with varying populations. These studies have demonstrated that food addiction does, in fact, exist in both normal weight and overweight subjects (Gearhardt, Corbin, & Brownell, 2009). However, in one study, having a food addiction was associated with a 3.5 fold higher risk of being overweight or obese (Pedram et al., 2013). In addition, subjects with food addiction have a higher rate of failed weight loss in both behavioral therapy and surgical populations (Davis et al., 2011).

This research may highlight a useful clinical tool for stratification and identification of obese patients who will require specific strategies to combat their addiction in contrast to similarly obese patients for whom food is not an addiction. A number of studies have suggested that 15% of patients needing behavioral treatment and 40% of patients seeking bariatric surgery for obesity met criteria for food addiction (Eichen, Lent, Goldbacher, & Foster, 2013; Meule, Heckel, & Kubler, 2012). Equally important, describing some foods as addictive reframes scientific understanding of control over food intake and shifts investigation toward the ways in which food affects the brain's reward system. Such investigations can take advantage of a large existing literature on addiction.

### **1.2.2 Shared Neural Circuitry Between Food and Drugs of Abuse**

The overlap between brain effects of drugs of abuse and over-eating have been demonstrated at the cellular level via shared changes in neuronal circuitry, as well as at the behavioral level in evidence from human imaging and survey studies. In 2007, the National Institute of Drug Abuse (NIDA) joined the NIH obesity task force to support research on the addictive properties of food. According to NIDA director Nora Volkow, "This research opens the door for us to apply some of the knowledge we have gathered about drug addiction to the study of overeating and obesity." That there are so many similarities between over-eating and addiction is not surprising. One of the principles of our understanding of drugs of abuse is that we are susceptible to these compounds because they manipulate the "pleasure" centers in the brain which have evolved to reinforce

behaviors that are good for us such as eating and sex. These behaviors release dopamine (DA) and trigger a series of neuroadaptations.

Dopamine is a key neurotransmitter in the limbic, or affective, systems of the brain. While this chemical has several complex functions, it is best known for its roles in movement disorders (such as Parkinson's disease) and reward signaling. The idea that DA is rewarding is partially informed by studies which demonstrate that animals will lever-press to stimulate DA neurons in the ventral tegmental area (VTA) or to obtain drugs that impinge on this system. In the natural setting, DA is released during food intake, sex, with unexpected gains such as in gambling—all deemed rewarding experiences (Hu, 2016). In one view, the reinforcing power of stimulating DA systems compels an animal to continue to seek out rewarding experiences, which, evolutionarily, were aligned with the animal's survival and reproductive success. Drugs of abuse hijack this system—sending it into overdrive by causing enhanced release of and/or response to DA. This DA surge is so rewarding and so motivating that the animal (or person) goes to lengths to repeat this experience. When engaging in rewarding behaviors continues despite negative consequences (e.g. financial loss, negative health impact, social isolation), this behavior is deemed to be 'addiction' (Konova, Moeller, & Goldstein, 2013; Willuhn, Wanat, Clark, & Phillips, 2010).

So, there is a close link between brain areas associated with food intake and with other rewarding behaviors and addiction. Now, as over-eating and obesity



have emerged as modern diseases, we can return to studying these circuits in their original context. At the same time, we can use insights from the longer history of addiction literature to inform our understanding of how over-eating is reinforced and how it may be controlled through behavioral or pharmacological interventions in the future.

As a biological process, addiction is characterized by perturbations in the affective and executive control pathways in the brain. While small amounts of DA release in response to food intake is good and leads to consistent and appropriate motivation to eat, the large releases caused by high-fat and high-sugar foods can establish a cycle of craving and dependence similar to that seen in addiction. Over the long-term, the same changes that are seen in the reward circuitry under the influence of cocaine and alcohol use can be demonstrated in animals and people who have prolonged intake of high-fat and high-sugar foods. One area of study in the field of addiction tracks changes in the DA receptors in individuals and animals with substance abuse. In 2001, a prominent study conducted by Wang et al. demonstrated that availability of the DA D2-receptor (D2R) is decreased in subjects with obesity (Wang et al., 2001), a finding that is also seen in subjects with addiction to cocaine (Volkow, Fowler, Wang, & Swanson, 2004; Wang, Volkow, Thanos, & Fowler, 2004). This finding is significant because deficiency of these receptors may result in dysfunctional responses to rewarding stimuli and promote enhanced drug or food intake. In support of this model, a 2010 study by Johnson and Kenny demonstrated that

the compulsive behavior exhibited by their high-fat fed obese rats correlated with low D2R expression. Moreover, viral knockdown of the D2R receptor accelerated weight gain and compulsive eating in high-fat fed rats.

With these studies, we can begin to build a framework for understanding the role of DA in promoting food intake. However, feeding behavior is complex. The decision of how much and when to eat integrates aspects of animal's motivation, body habitus, hormonal state, and perceived value of food. The role of DA in moderating this behavior is similarly complex and so efforts to understand these dynamics are helped by narrowing our focus to a one or two DA-responsive brain regions.

### **1.3 Food Intake and the Nucleus Accumbens**

A more basic understanding of how reward system activity patterns govern food intake is required to take the next steps in designing both behavioral and pharmacological treatments. Study of the Nucleus Accumbens (NAc) has contributed to our understanding of neural mechanisms underlying motivation to seek out and consume high calorie food. The NAc lies within the ventral striatum, or limbic division, of the basal ganglia, and its critical functions include processing information about rewarding stimuli and directing behavior to maximize attainment of reward (Humphries & Prescott, 2010; Wickens, Budd, Hyland, & Arbuthnott, 2007). The role of the NAc in food reward and food intake

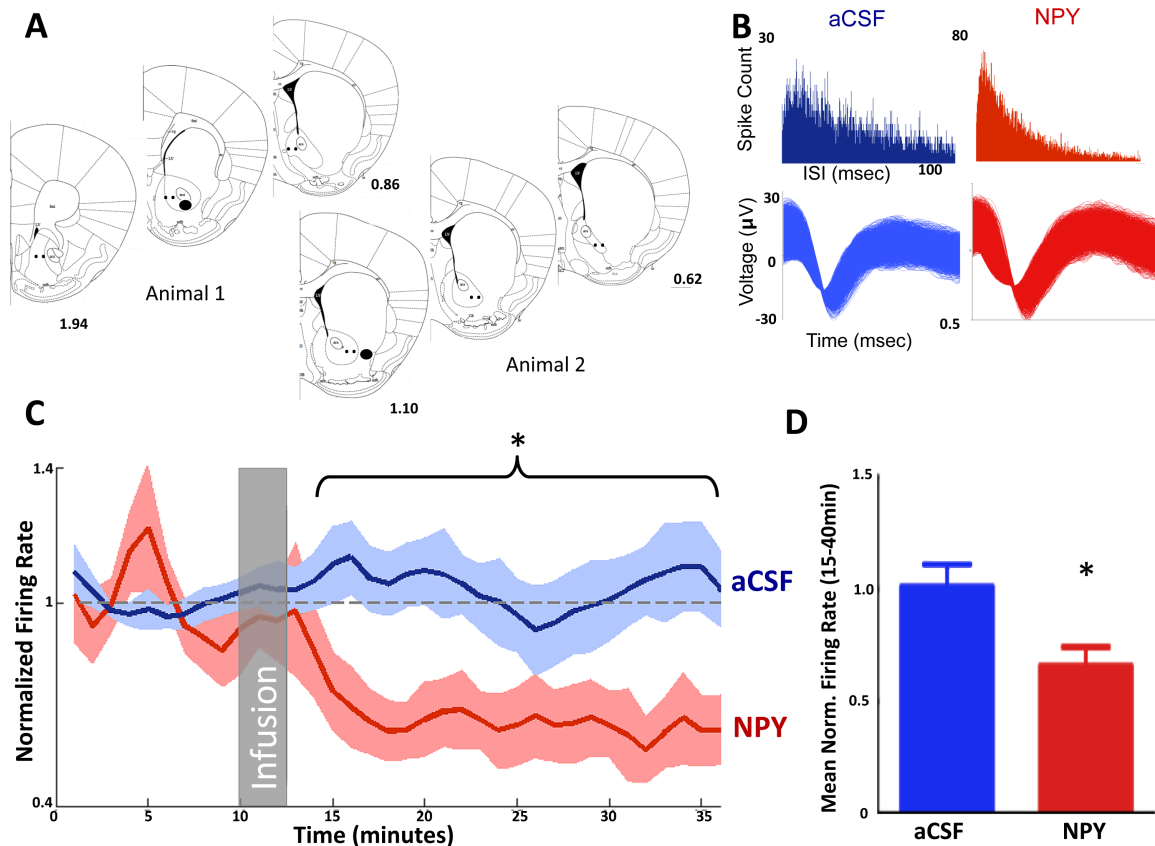
has been the subject of much study (Kelley, 2004). The NAc is involved in promoting consumption of palatable (rewarding) foods, which, includes those that are calorically dense—high in fat and/or sugar. Much of this work was established by Ann Kelley, who demonstrated the importance of NAc projections to the lateral hypothalamus (LH, known as a “feeding center”) in reinforcement of food intake (Maldonado-Irizarry, Swanson, & Kelley, 1995; Stratford & Kelley, 1999).

As noted above, DA is a key neurotransmitter that is released in the NAc in response to rewarding stimuli, including food (Bassareo & Di Chiara, 1999; Hajnal & Norgren, 2005). Enhanced DA release in the striatum has been implicated in disorders of pathological food intake such as binge eating disorder (Avena et al., 2008; Wang et al., 2011), a disorder defined by rapid consumption of large amounts of food and loss of control over eating (DSM V).

Previous studies have demonstrated that inhibition of NAc neurons promotes food intake (Stratford, Swanson, & Kelley, 1998). For example, generalized inhibition of NAc activity with GABA agonists such as muscimol and baclofen causes animals to increase food intake dramatically (Stratford & Kelley, 1997). Conversely, electrical stimulation of neurons within NAc inhibits food intake (Halpern et al., 2013; Krause, German, Taha, & Fields, 2010). Evidence suggests that these general effects are mediated by a subset of striatal neurons (Taha & Fields, 2005, 2006). It has also been demonstrated that striatal neurons exhibit one of two stereotyped responses to food intake (Krause et al., 2010).

The majority of recorded neurons, Type 1 neurons (~60%), are inactivated prior to initiation of sucrose consumption. These neurons inactivate approximately 2 seconds before initiation of licking behavior. A second population of neurons, labeled Type 2 (~30%), respond to the hedonic value of food and increase firing rates in response to higher concentrations of sucrose, as compared to low sucrose concentrations or water.

Presence of inhibited Type 1 neurons is also observed after infusion of the pro-feeding peptide, NPY. When injected into the ventral striatum, NPY preferentially increases consumption of fat and sugar-containing foods over regular chow (Pandit, la Fleur, & Adan, 2013). In previous work in our lab, I conducted recordings of NAc neurons after NPY infusion which demonstrate general inhibition (Fig 1) (van den Heuvel et al., 2015), consistent with the model of reduced firing.



**Fig 1. Neuropeptide Y (NPY) in the nucleus accumbens decreases neuronal activity in vivo.** (A) Location of electrodes (black spots) and cannulae (filled black circles) projected on a mouse brain atlas section. (B) Interspike interval (ISI) histograms and waveforms from a single unit in artificial cerebrospinal fluid (aCSF) infusion sessions (left) and the same neuron in NPY infusion sessions (right). (C, D) Average neuronal activity for NPY sessions and aCSF. Gray box represents time of infusion. NPY significantly reduces firing rate from 15 minutes to 40 minutes. np , .05. Norm., normalized.

This effect is largely the result of significant suppression in the firing of a subset of recorded neurons (~68%), a similar proportion seen in studies of Type 1 neurons. There is a gap in knowledge, however, linking the activity of specified subsets of dopamine-responsive neurons (expressing D1 and D2 receptors) in

the NAc to observed activity patterns during feeding behaviors. To date, it has been difficult, if not impossible, to conduct recordings of specific neuronal sub-populations (i.e. D1R and D2R –containing medium spiny neurons) in deep brain areas such as the ventral striatum. In 2014, a scientific breakthrough, called fiber photometry, made such investigations feasible. Here, we employ this novel method of population neural recording to assess activity patterns of D1R, D2R and VTA DA neurons during various feeding behaviors.

## 2 Statement of Purpose

Previous studies of the role of the NAc in directing feeding behavior have demonstrated two main findings: first, inhibition of the NAc is sufficient to initiate feeding, and second, a smaller subset of NAc neurons encode the reward value of what is ingested. These functional classes of neurons have been termed Type 1 (inhibitory) and Type 2 (excitatory) respectively (Taha & Fields, 2005).

Because the medium spiny neurons (MSNs) of the NAc send GABAergic projections to various brain regions including the lateral hypothalamus (LH, feeding center) and the ventral tegmental area (VTA, reward center), it has been hypothesized that inhibition of these neurons results in release of inhibitory input on downstream feeding and reward-seeking circuits (Stratford & Kelley, 1999). Anatomical mapping of NAc projections to LH and VTA has demonstrated that these primarily consist of D1R+ MSNs. And activation of this circuit interrupts food intake behavior (Krause et al., 2010; O'Connor et al., 2015). Taken together, data suggest that D1R MSNs are those inhibited during food intake (Type 1). However, a second body of literature would suggest that D1R MSNs are also reward responsive (Calipari et al., 2016).

Fiber photometry recordings of D1R MSNs during various feeding behaviors can help resolve this discrepancy. Based on projection patterns and previous manipulations of D1Rs, we hypothesize that D1Rs will exhibit Type 1 inhibition to promote food intake. It is also possible, however, that a separate population of D1Rs are capable of exhibiting Type II excitation in response to palatable food.

We will also explore the activity patterns of D2R MSNs and VTA neurons in food intake as we begin to build a more complete understanding of reward system responsiveness to feeding.



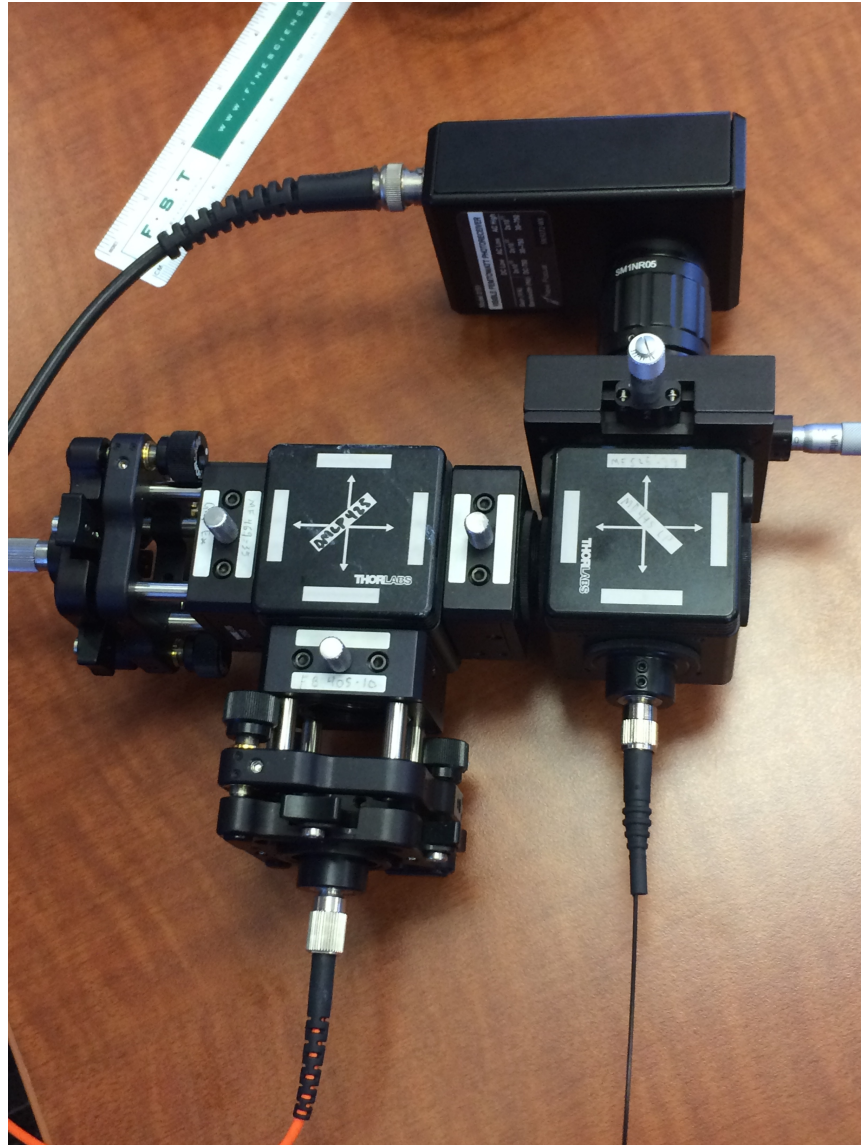
### 3 Methods

#### 3.1 Fiber Photometry

Fiber photometry is a powerful new technique for evaluating neuronal firing *in vivo* by using GCaMP expression in select neurons, and simultaneous excitation and monitoring of fluorescent light emission via a fiber optic cable (Gunaydin et al., 2014). Intracellular Ca<sup>++</sup> sensors are commonly used to detect action potentials and assess neuronal activity. The GCaMP family of Ca<sup>++</sup> sensors emit fluorescence when intracellular Ca<sup>++</sup> is high (Akerboom et al., 2012; Bootman, Rietdorf, Collins, Walker, & Sanderson, 2013; Dreosti, Odermatt, Dorostkar, & Lagnado, 2009). The intensity of this fluorescence scales linearly with number of action potentials (Y. Chen, Lin, Kuo, & Knight, 2015). Historically, GCaMP has been used to capture spiking activity of small sets of superficial neurons that can be assessed visually. Fiber photometry uses a femtowatt photodetector to capture fluorescence from relatively large populations of cells expressing a Ca<sup>++</sup> sensor in deep brain structures. This population fluorescence is collected through an optical fiber that delivers excitation light to the labeled tissue and simultaneously relays emitted fluorescence to a photo-detector (Cui et al., 2014; Cui et al., 2013; Gunaydin et al., 2014).

The goal of fiber photometry is to conduct recordings of native activity of a highly specified set of neurons. Because GCaMP can be delivered to the brain area of interest using a viral vector, the population of cells recorded can be limited to an area that is approximately 500 $\mu\text{m}^2$ . Further neuronal specificity can

be achieved using Cre-driver lines to drive expression of the Cre-recombinase enzyme in selected cell-types (i.e. D1-cre and D2-cre animals in the present study). GCaMP virus that is “floxed” is only active in the presence of Cre-recombinase. We have developed an independent setup for these recordings in our lab (pictured below), and have successfully recorded activity in D1R and D2R medium spiny neurons in the NAc during instrumental behavior. For fiber photometry, we use 490nm LED excitation light (Thorlabs) that is passed through a series of collimators and cleanup filters and finally into a patch cord (Doric). A Cre-dependent GCaMP6s is delivered surgically into the NAc or VTA. A 400  $\mu\text{m}$  optical cannula is then implanted targeting the region of interest (NAc, VTA cell bodies, or terminals). For the behavior, a patch cord is connected to an indwelling optical cannula and the mice are allowed to move freely. LED stimulation is controlled through a computer program that modulates a sinusoidal 531Hz signal through an LED driver that can accommodate up to 4 separate channels. A second, control signal, 405 nm LED is modulated simultaneously at 211Hz and delivered through the same patch cord and into the optical cannula. This 405 nm signal does not excite GCaMP and so, serves as a control for changes in auto-fluorescence and background noise in the data.



The fluorescence data is then collected along with timestamps for animal activity, nose pokes, and magazine entries. A strong advantage of this technique lies in the ability to evaluate real-time changes in neuronal firing rates in awake, freely moving mice.

### 3.2 Animal Surgeries and behavioral training

Animals: Twenty-seven D1 and D2-dopamine receptor Cre-recombinase male and female mice (*Drd1a-cre<sup>+</sup>* and *Drd2a-cre<sup>+</sup>* strain EY262, Gensat, backcrossed at least 10 generations to a C57Bl/6 background) weighing 20–30 g were used for these studies. All animals were group-housed until surgical cannula implantation when individuals were single-housed. Animals were on a 12 h light/dark cycle and provided standard chow and water *ad libitum* except during behavioral training described below, and all animal procedures were performed in accordance with the protocol approved by the Institutional Animal Care and Use Committee (IACUC).

Behavioral Training: *Sucrose Licking* Behavioral boxes (MedAssociates) have been set up to accommodate free access to a sipper. To encourage licking, animals are water deprived overnight before the first day of training. Animals are then given free access to a 5% or 10% sucrose solution. After training day 1 animals are not food or water restricted. Individual licks on the sipper are recorded. For fiber photometry recordings, licks register in real time together with fluorescence. *Pellet Eating* Behavioral boxes (MedAssociates) have two nosepekes on either side of a pellet receptacle. Animals were chow restricted to maintain 85-90% of bodyweight. Before the first day of training, animals are exposed to purified pellets in their home cages. During sessions, a nosepoke in the correct port results in delivery of a pellet after a 3 second delay while

nosepokes in the incorrect port elicits no action. There is no limit to the number of pellets that may be earned. These sessions are videotaped for later behavioral coding. Videos are analyzed to determine latency between pellet drop and magazine entry, pellet retrieval time, and trial errors that include ignored pellet or dropped pellet.

Surgical procedure: Animals were anesthetized with an induction dose of ketamine (100 mg/kg) and xylazine (10 mg/kg) and maintained with supplemental doses of ketamine (30 mg/kg) every 45-60 minutes. The skull was leveled between bregma and lambda landmarks, and a single craniotomy was performed over the ventral striatum or ventral tegmental area. First, 0.5  $\mu$ l of the floxed GcAMP6s virus was injected at AP: +1.4, ML:  $\pm$ 0.7, DV: -4.5 (relative to bregma) to target D1R and D2R NAc neurons. VTA dopamine neurons were targeted for injection at AP: -3.5, ML  $\pm$ 0.35, DV -4.8. Next, a 400  $\mu$ m fiberoptic cannula (Doric) was placed in the same craniotomy. Cannulas were affixed to the skull with dental cement and a screw placed over the parietal bone. After surgery, all animals are single-housed to prevent cage-mates from damaging each other's cannulas.

Fiber photometry recordings: After approximately 2-3 weeks of recovery to ensure optimal viral expression and neuronal signal, animals were recorded during either the sucrose licking or operant pellet task. Plugging and unplugging

from the fiber optic patch cord was performed under isoflurane anesthesia to minimize stress on the animals.

Viral Vectors: AAV-Ef1a-DIO-GcAMP6s virus for Fiber Photometry was purchased from UPenn vector core.

*Author's Contribution: All methods, surgeries and procedures described above (including assembly of FP rig) and statistical analyses that follow were conducted solely by this author.*

### **3.3 Statistical methods and analysis**

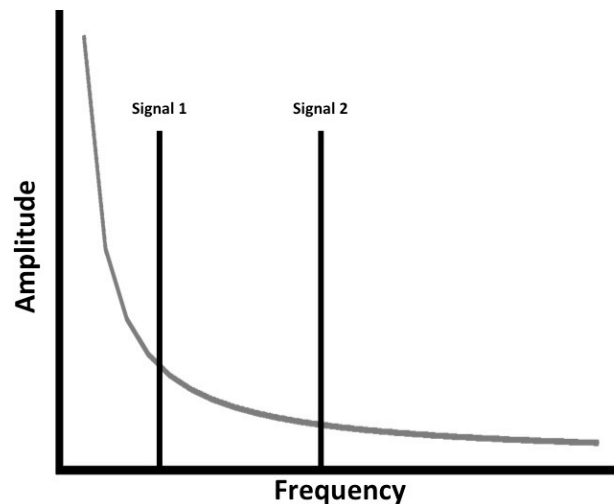
The primary goal of fiber photometry analysis is to make sense of a complex signal. This is achieved experimentally by using modulation frequencies that maximize signal to noise ratio, and mathematically by careful demodulation of the experimental signal such that fluorescence changes are recovered in full.

#### Modulation of Excitation signals

*The purpose of signal modulation is 2-fold*

1. Modulation allows recovery of a signal that is specific to the excitation light and that excludes other sources of fluorescence or noise.

2. Modulation allows the researcher to pick a frequency for the excitation signal that is away from significant sources of noise that may obscure true signal and thereby increases SNR (Fig 2).



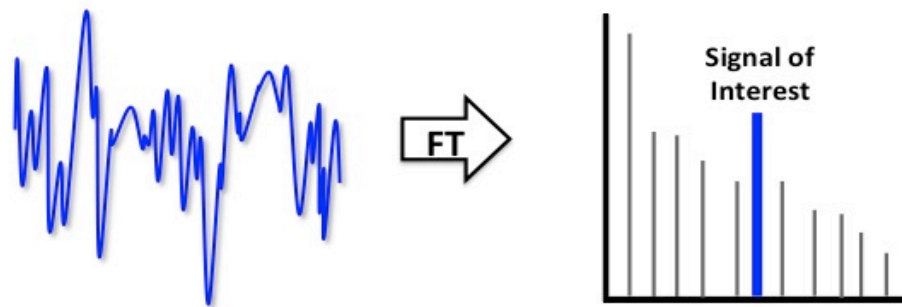
**Fig 2. Choosing modulation frequencies.** Example noise band  $1/f$  demonstrates decreasing amplitude of noise envelope as frequency gets larger. Two example signals 1 and 2 have the same amplitude but are differently obscured by noise. Signal 1  $S:N \cong 3/1$ ; Signal 2  $S:N \cong 6.5/1$

In our set-up, modulation is achieved through the TDT RPvdsX software that sends out a sine wave of specified frequency and amplitude to the LED driver to drive both the excitation light (531Hz) and the background (control) light (211Hz). Modulation parameters are chosen such that sources of noise (e.g. electrical noise (60Hz), and fluorescent lights (120Hz)) and their harmonics are avoided. The excitation and background modulation frequencies should also be chosen to minimize harmonics between the two.

#### Demodulation of excitation signals

A signal that is modulated to a low-noise frequency can theoretically be recovered simply by applying a narrow filter to the raw (observed) signal of

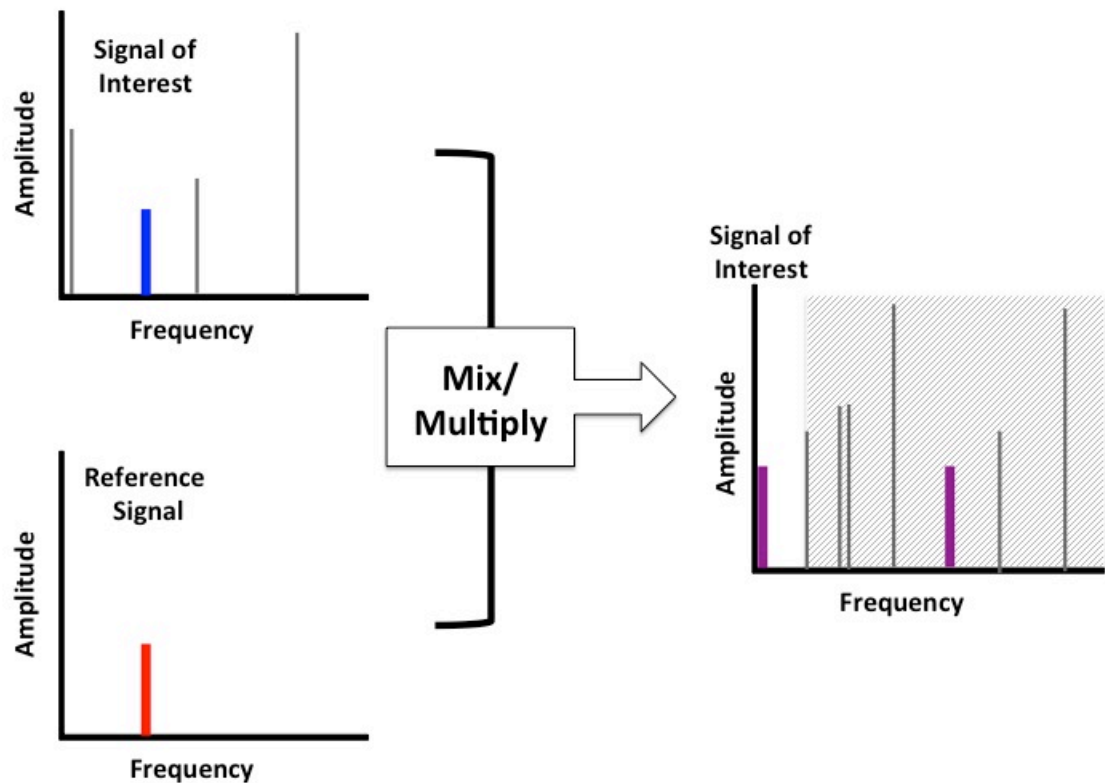
detected fluorescence that removes all frequencies that are not at the modulated frequency (plus some small bandwidth). However, it is “challenging to design very narrow, (high Q) band-pass filters with discrete components. If the specifications call for an extremely narrow filter, it may even be impossible.” (Orozco, 2014)(Fig. 3).



**Fig. 3. Fourier transform of observed raw signal demonstrates a peak at the chosen modulation frequency.** Even though signal to noise ratio is acceptable, there are significant sources of noise at frequencies close to the modulation frequency. It would be challenging to design a bandpass filter to recover only this frequency and exclude nearby noise.

Demodulation allows for recovery of signal using a simple low-pass filter by moving signal of interest to DC frequency (0Hz). (Fig 4)



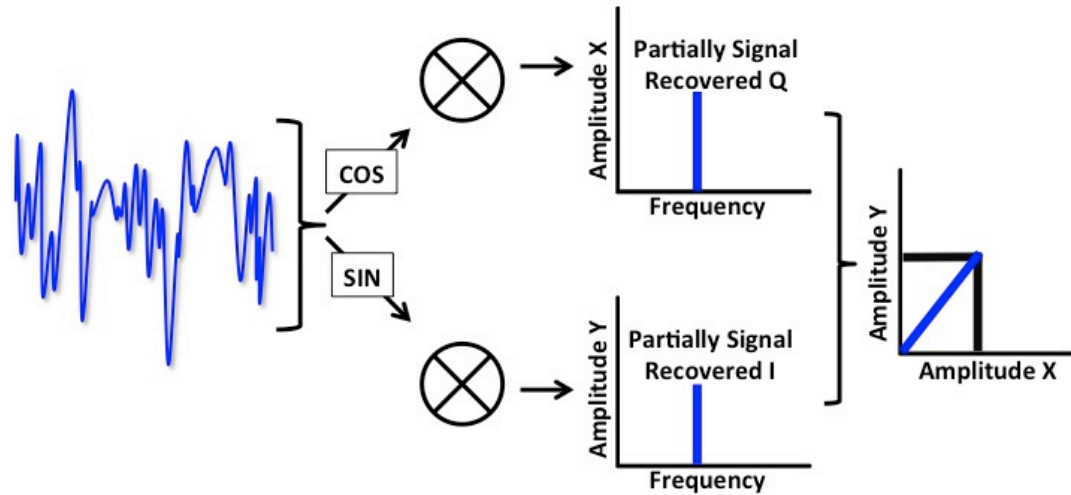


**Fig 4. A pure reference signal is mixed (multiplied) with the observed raw signal.**

Fourier transform of the raw signal demonstrates a peak at the experimental modulation frequency of interest (thick band, 1kHz) but also demonstrates large amplitude noise at 50Hz, 1.5kHz and 2.5kHz. Mixing a pure 1kHz reference signal creates double peaks at each observed frequency that are equal to the reference frequency  $\pm$  each observed frequency. As a result, our experimental signal moves to zero and all other sources of noise move away from zero. This makes recovery of the experimental signal easy through a simple low-pass filter.

To recover the full excitation signal accurately, a reference signal is mixed with the measured signal to demodulate. Reference signal is mixed both in-phase and out-of-phase and then these components are added in quadrature to recover

any loss of signal that occurs from phase shifts between reference signal and modulated (experimental) signals (Fig. 5)



**Fig 5. Mixing with a reference signal and adding in quadrature.** To account for shift in phase between reference and recovered signal (which will commonly happen if there are any filters in the input pathway, for example). Reference is mixed both in-phase and out-of-phase to give full amplitude of experimental signal. This is called, adding in quadrature I.Q. and comes from the Pythagorean theorem.

So, for demodulation, we apply the following equation to mix a reference signal with our observed raw signal (Eq. 1).

$$A \sin(2\pi f_m t) \times B(\sin(2\pi f_m t)) = \frac{1}{2} AB - \frac{1}{2} AB \cos(2\pi 2f_m t)$$

Where A = experimental signal, B = reference signal,  $f_m$  = frequency of modulation. Then, to account for phase shifts,

$$\text{Exp}_{\text{sig}} \times \text{Ref}_{\sin(\text{sig})} = I$$

$$\text{Exp}_{\text{sig}} \times \text{Ref}_{\cos(\text{sig})} = Q$$

$$\text{Exp}_{\text{sig}} = \sqrt{I^2 + Q^2}$$

This process is repeated using the same raw observed signal referenced against the modulation frequency of the background (control) signal to recover background fluorescence during experimental trials.

Finally, a low-pass filter is applied  $\sim 15\text{Hz}$  during live collection and later further filtered at  $2\text{Hz}$  during post-processing to recover both GCaMP-related and background fluorescence signals.

#### Normalizing Fluorescence signal

dF/F can be calculated in a number of ways, and consensus still has not evolved in the literature. For the following experiments, we use the recovered 490 nm (excitation) signal relative to some baseline local to an individual trial or across whole experimental session (depending upon how much bleaching occurs)

$$490\text{nm signal/baseline}$$

dF/F can be calculated by referencing the normalized 490 nm signal against the normalized 405 nm (background) signal that is used in this set-up.

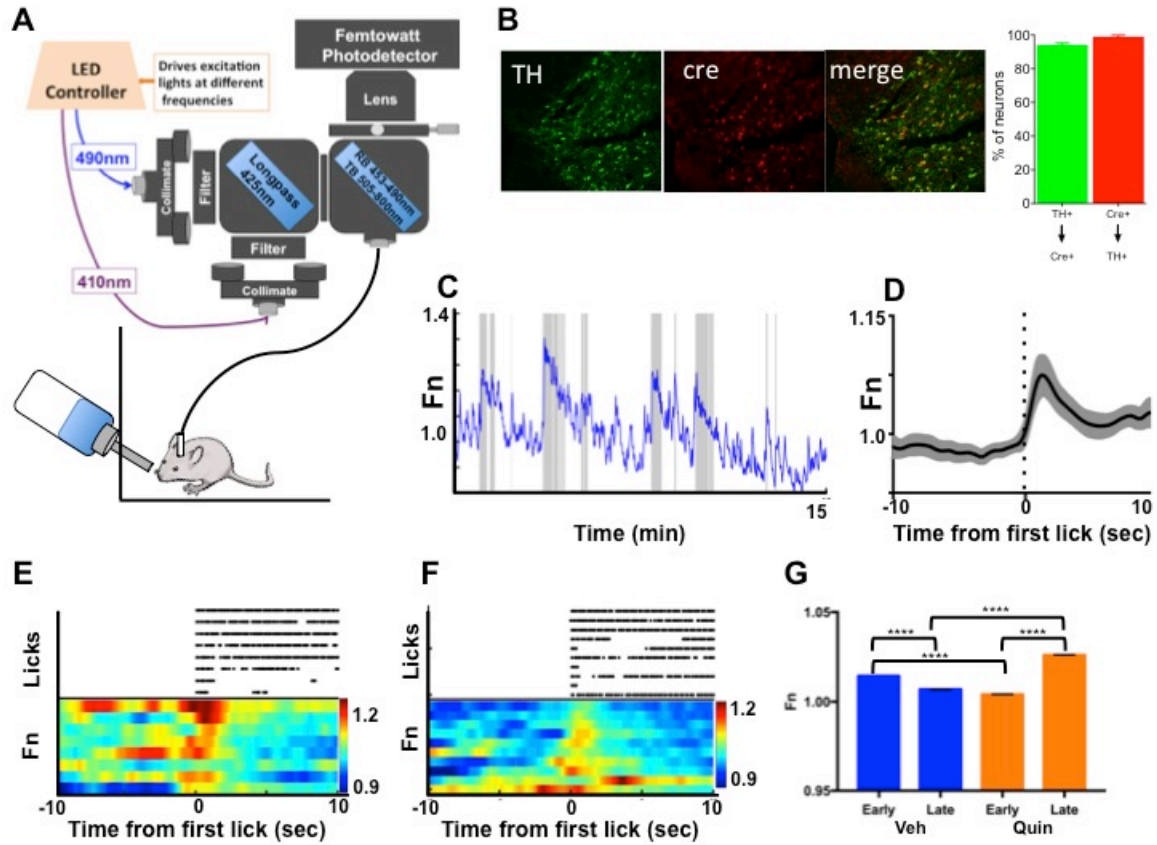
$$490\text{nm signal}/405\text{nm signal}$$

To account for bleaching, the 405nm signal can be fitted to a linear least-squares regression line so dF/F becomes:

$$dF/F = (490 \text{ signal} - \text{fitted } 405 \text{ signal})/\text{fitted } 405 \text{ signal}$$

## 4 Results

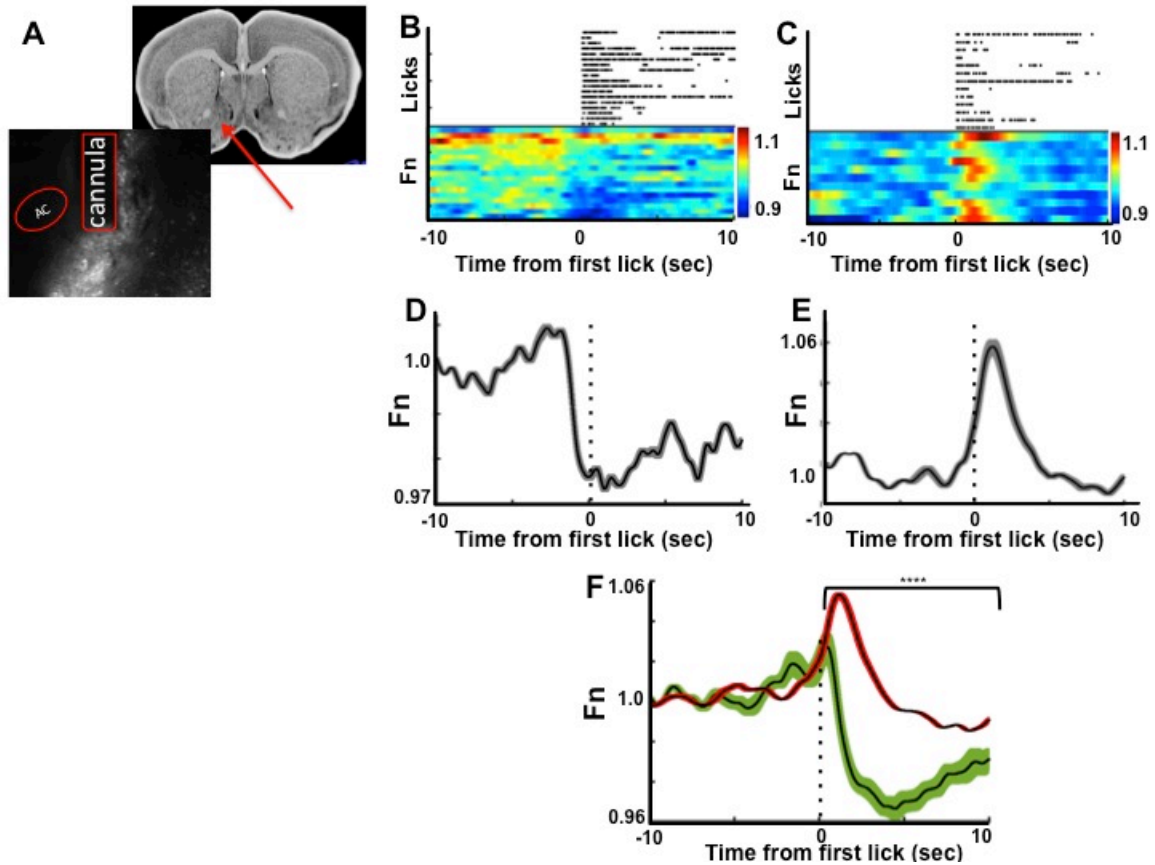
My first aim was to establish the fiber photometry technique and confirm that it works. To do this, D2-Cre animals expressing Cre recombinase in VTA-TH neurons (that release dopamine, Fig. 6B) were infused with virus to express GCaMP6s and fitted with a fiber-optic cannula. VTA-TH neurons are dopaminergic and release DA in the NAc in response to palatable food intake. Recordings of DA-neuron activity during sucrose licking demonstrate large increases in firing in response to 10% sucrose ingestion (Fig. 6C-D,  $n = 11$ , average normalized fluorescence (aFn) peak = 1.7 at lick onset). These increases in firing were concentrated at the beginning of lick bouts and were somewhat attenuated over time (Fig. 6E,G  $aFn_{\text{start}} = 1.015 \pm 1.0280e^{-4}$  in first four lick bouts compared to the last four  $aFn_{\text{end}} = 1.007 \pm 0.7449e^{-5}$ ,  $p < 0.0001$  by one-way ANOVA). To test sensitivity of fiber photometry to changes in population activity further, animals were injected with a low-dose D2-agonist, quinpirole (0.001mg/kg administered i.p.). Because the D2R is  $G_i$ -coupled, firing of VTA-DA neurons is attenuated in the beginning of the trial. As quinpirole wears off, VTA firing strengthens, in contrast to attenuation seen during earlier sucrose trials (Fig. 6F,G  $aFn_{\text{veh}} = 1.007 \pm 0.7449e^{-5}$  in last four lick bouts compared to  $aFn_{\text{quin}} = 1.026 \pm 1.8842e^{-4}$ ,  $p < 0.0001$  by one-way ANOVA).



**Fig. 6 Activity of VTA dopamine neurons during sucrose licking.**

(A) Representative image of fiber photometry set up, with animal tethered during recording session. (B) VTA neurons expressing TH (marker of dopamine synthesis) also express D2 receptor as indicated by the presence of Cre recombinase in these cells (D2-cre animal, 95% TH+ neurons express Cre, 100% of Cre+ neurons are also TH+). (C) Example raw fluorescence trace (in blue) during 15 minutes of licking (gray bars indicate individual licks). (D) Mean and s.e.m. of normalized signal for VTA animals during sucrose licking demonstrates sharp increase in activity after first lick (dashed line) of analyzed lick bouts ( $n = 12$  animals, 229 bouts). (E, F) Example 1 hour licking sessions for the same VTA animal after i.p. injection with saline (E) and quinpirole (F). Top panel: Individual licks were binned into clustered bouts (defined by at least 5 consecutive licks occurred within 1 sec) and then arranged by bouts separated by 10 seconds to provide a clean baseline for analysis of fluorescent signal ( $n=9$  and 10 bouts for veh and quinpirole examples, respectively). Bottom panel: heatmap of normalized signal intensity aligned to lick bouts such that each line corresponds to one lick bout. (G) Mean and s.e.m. of normalized signal for the first four lick bouts vs last four lick bouts of each session demonstrates significant attenuation of signal under normal conditions is reversed when D2-agonist quinpirole is onboard ( $p < 0.0001$ , one-way ANOVA).

Having established reliable recordings under various conditions, we next examined activity of D1R and D2R NAc MSNs during sucrose licking. As expected, D1R and D2R neurons demonstrate opposite activity patterns during sucrose licking. D1R neurons shut down immediately prior to lick onset (Fig. 7 B,D prelick  $\Delta F_{D1} = 0.005 \pm 0.00015$  compared to postlick  $\Delta F_{D1} = -0.0338 \pm 0.00036$ , two-tailed t-test,  $p < 0.0001$ ). As hypothesized, this pattern of activity is consistent with the Type 1 pattern previously described in NAc neurons (Krause et al., 2010; Taha & Fields, 2005). In contrast, D2Rs demonstrate a strong increase in signal at lick onset (Fig. 7C,E peak  $F_{D2} = 1.0632 \pm 0.0011$ ). This pattern of activity is consistent with the Type 2 excitatory pattern of activity.



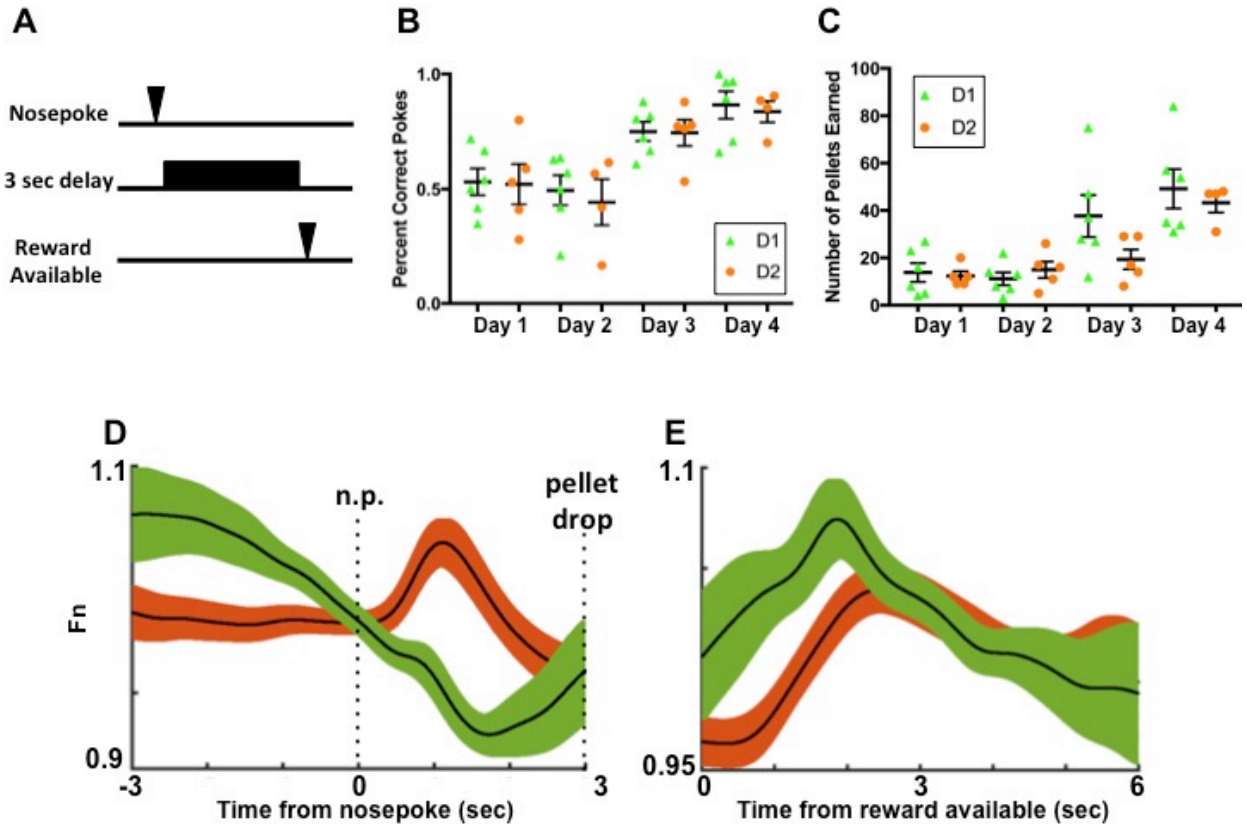
**Fig. 7 Differential activity of D1R and D2R neuron activity during sucrose licking.**

(A) Representative image of cannula placement and GCaMP6s viral expression in the NAC. (B,C) Example 1 hour licking session for a D1(B) and D2(C) animal. Top panel: Individual licks were binned into clustered bouts (defined by at least 5 consecutive licks occurred within 1 sec) and then arranged by bouts separated by 10 seconds to provide a clean baseline for analysis of fluorescent signal (n=17 and 14 bouts for D1 and D2 examples, respectively). Bottom panel: heatmap of normalized signal intensity aligned to lick bouts such that each line corresponds to one lick bout. (D,E) Mean and s.e.m. of normalized signal for example sessions depicted in B and C. (F) Mean and s.e.m. of normalized signal for all D1 and D2 animals first day of sucrose licking (For D1, n = 4 animals, 66 bouts. For D2, n=2 animals, 32 bouts.  $p < 0.0001$ , two-tailed t-test).

Studying sucrose licking allows us to relate FP data to electrophysiological studies of Type 1 or Type 2 neurons, however this single behavior overlaps several aspects of feeding (e.g. approach, decision, initiation, intake, reward, and cessation). To understand better the roles of D1R and D2R neurons in these distinct aspects of feeding behavior, we trained animals on an operant feeding task in which they nose-poked for pellets. This task allows us to separate the decision and action initiation phases of intake from reward receipt for more in-depth analysis.

Analysis of poke and reward-phase fluorescence on last day of training, when animals had adequately learned the task (Fig. 8B,C) demonstrates that D1R and D2R neurons continue to have opposite patterns of activity at action-initiation during the poke phase of each trial (Fig. 8D). However, when reward-phase fluorescence is considered separately, both D1R and D2R neurons have increases in activity associated with food intake (Fig. 8E).





**Fig. 8 Differential activity of D1R and D2R neuron activity during operant pellet eating task.** (A) Schematic of operant pellet task. Nosepoke in the correct port results in delivery of purified sweet pellet 3 seconds later. (B) Learning curves for both D1 (n=6) and D2-cre (n=5) strains. In first 2 days of training, animals poke equally in the active and inactive noseports. By Day 3, however animals have learned which port is the active (correct) one and pokes in the incorrect port decline significantly (mean+s.e.m Day 1 compared to Day3,  $p < 0.008$ , by one-way ANOVA). Individual animal data points plotted along with mean and s.e.m. (C) Over 4 days of training, food restricted animals earn more pellets as they learn that correct nosepokes result in pellet delivery. There is no limit on number of pellets earned. On average, animals of both strains earn and eat 45 pellets per 1 hour session. (D) Analysis of the nosepoke phase of repeated trials on day 4 of training demonstrates significant difference in activity pattern of D1R and D2R neuron populations. (For D1, n = 6 animals, 290 trials. For D2, n=5 animals, 203 trials.  $p = 0.0072$ , two-tailed t-test.). (E) Analysis of the reward phase of repeated trials on day 4 of training demonstrates significant difference in activity pattern of D1R and D2R neuron populations. (For D1, n = 6 animals, 290 trials. For D2, n=5 animals, 203 trials.  $p < 0.0001$ , two-tailed t-test).

## 5 Discussion

The present study accomplishes two aims. First, we have validated a new technique, fiber photometry, to conduct cell-type-specific recordings of neuronal activity of medium spiny neurons in the nucleus accumbens. Second, we build on literature assessing the roles of NAc D1R and D2R neurons in food intake. Earlier studies have suggested differential roles of D1R and D2R neurons based on anatomical tracing, pharmacological and optogenetic manipulation of these cells. However this is the first direct observation of these populations during food intake.

Our D1R results are consistent with earlier hypotheses that these neurons gate intake behaviors (O'Connor et al., 2015). In both the licking and nosepoke paradigms, D1R activity is rapidly and significantly attenuated at the start of each trial. During sucrose licking, D1R suppression is reliably sustained throughout the lick bout and signal rebounds after the final lick of each bout. These findings are consistent with the patterns of activity previously described in electrophysiological recordings of NAc gating neurons originally described by in 2005 (Taha & Fields, 2005). This suggests that the previously characterized neurons might be D1R expressing cells.

There is a slight discrepancy, however, between our fiber photometry recordings and electrophysiological recordings of Type 1 neurons in that Type 1 neurons classically become inhibited approximately one second before licking

onset. While this pattern held in some examples (Fig. 7D), on average, we found that, decreases in activity occurred right on the first lick (Fig. 7F). This discrepancy is likely due to a difference in technique. Electrophysiological recordings have a time resolution of 1-2 ms and so changes in neuronal activity are accessed immediately. We used GCaMP6s (“6” indicates this is the sixth generation of GCaMP molecules, “s” stands for slow) for our fiber photometry recordings. Although there are faster versions of the GCaMP6 molecule, we opted for 6s because it allows it has the best signal to noise ratio, and largest change in fluorescence per action potential. However, GCaMP6s also has a longer rise time (approx. 500 ms) and decay time ( $T_{1/2} = 3$  s for 100 action potentials) than other GCaMP6 molecules (T. W. Chen et al., 2013). So, while we have maximized our signal using this construct, there is a potential delay between changes in signal and real-time behavior of up to 500ms at onset and 1-2 seconds at offset. Because we do not know what the exact correction would be in each animal (it may vary with size of infection and number of neurons captured) we have not attempted to compensate for these small time-shifts in fluorescence data relative to behavioral data.

In contrast to licking behavior, the operant pellet task allowed us to assess action initiation and reward retrieval as discrete timepoints. This paradigm also keeps the size of reward constant (1 pellet), whereas in free licking the animal is in control of lick bout size and spacing between bouts. With this task, inhibition of D1Rs did anticipate action initiation by 1-2 seconds (Fig. 8D). Reasons for this

difference are not clear. It could be that more motor coordination and planning is required to accomplish a nosepoke than a lick and so pre-poke inhibition begins earlier. In order to reduce the number of accidental nosepokes when the animal is exploring the noseport, our nosepoke sensor is recessed such that a deep, somewhat sustained ( $> 300\text{ms}$ ) poke is required to earn a pellet.

The operant pellet model also differs from sucrose licking by dissociating motor action from reward receipt and introduces a delay between the two. Under these conditions, we see D1R signal begin to return to baseline before reward is delivered and peaks immediately after the animal retrieves the food pellet (Fig. 8D,E). In this case, it is difficult to say whether the D1R signal increase after pellet retrieval represents a return to baseline after previous suppression or excitation due to reward. Alternatively, because these are population recordings, we may be seeing the activity from two separate sub-populations of D1R neurons. One set (the Type 1), undergoes sustained inhibition at the onset of each trial while a second set of D1R neurons may increase activity in anticipation of reward and peak after reward receipt –a pattern more similar to Type 2 neurons. In this case, these neurons should be sensitive to timing of reward delivery as well as value of reward delivered. Further studies are necessary to resolve this question, however, here we have established an appropriate paradigm for testing reward sensitivity independent of action initiation.

Our hypotheses about activity patterns of D2R MSNs during intake were less clear, due to lack of consensus in the literature about the role these neurons.

The D2 receptor is  $G_i$ -coupled and so, classically, when dopamine is high (as it is in response to food intake) these neurons are thought to be inhibited. Recent data challenge the classical understanding of the roles of D2 in reward and motivation. In 2010 Cui et al. demonstrated that in the dorsal striatum, some D2s are activated with action initiation (Cui et al., 2013). As mentioned previously, studies of D2 receptor availability suggest that obesity is associated with a decrease in the number of D2 receptors in the striatum (Johnson & Kenny, 2010; Wang et al., 2001). Loss of the inhibitory D2 receptor should lead to greater neuronal excitability and thus, according to these data, increased feeding would be associated with increased D2R neuron activity.

Our investigation of D2R MSNs suggests that these neurons correspond to Type 2 (excitatory) neurons found by Taha and Fields. These neurons are reward responsive and peak approximately one second after the start of sucrose consumption (Fig. 7E,F). Similarly, during the pellet task, D2R neurons peak after pellet delivery (Fig. 8E). Of note, video analysis of pellet task sessions demonstrates that well trained animals retrieve the food pellet almost immediately and so the peak seen after pellet delivery is associated with ingestion, not just reward receipt. Our analysis also demonstrates a peak in D2R neuron activity after nosepoke (Fig. 8D). The NAc is an important center for reward-associated learning and several studies have demonstrated that neurons in this structure respond to reward-predictive cues. Thus far, it is unclear whether the D1R or D2R population of neurons is more responsive to predictive

cues. In 2016, Soares-Cunha et al. demonstrated that activation of D2 neurons during reward-predictive cue enhanced lever pressing (Soares-Cunha et al., 2016). The present study provides further evidence that activation of D2R neurons encodes information about motivation and reward.

In sum, observing the activity of D1R and D2R neurons of the NAc fills gaps in our knowledge of normal regulation of these sets of neurons during appetitive and consummatory behavior. This is critical for developing a more accurate model of what this key region does in ingestive and reward-related behavior.

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